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COMMENTARY

Excitation–Contraction Coupling

Orai1 as a potential "fits-all approach" therapeutic target for the treatment of DMD

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Duchenne muscular dystrophy (DMD) is an X-linked disorder caused by loss-of-function mutations in the dystrophin gene. DMD is a progressive disease that affects 1 in 3,500–5,000 male births and results in muscle weakness, respiratory and cardiac failure, and, in due course, premature death commonly in the third decade of life (Mendell and Lloyd-Puryear, 2013). Histopathological hallmarks of DMD muscles include central nucleation, fibrosis, inflammation, and muscle fiber pseudohypertrophy amongst others (Zweyer et al., 2022). The dystrophin gene was discovered in 1987 (Hoffman et al., 1987), and since then there have been numerous efforts to develop therapies to delay disease progression and enhance muscle function in DMD patients.

Present widespread therapeutic recommendations include corticosteroids, cardiac medications, and, eventually, assisted ventilation to slow down disease progression, reduce symptoms, and improve quality of life, but do not target the underlying mechanism of the disease. However, pharmacological advances using oligonucleotide-mediated exon skipping to bypass variant exons and gene editing to provide truncated forms of dystrophin have gained interest in recent years, and several clinical trials are under way to assess their efficacy (Eser and Topaloğlu, 2022). Although these are promising advancements, there are still challenges to overcome and room for improvement in the area. For example, the TREAT-NMD DMD global database contains over 7,000 dystrophin mutations (Bladen et al., 2015), raising significant challenges for the development of gene correction therapies that might be applicable to large cohorts of patients rather than single individuals. Moreover, present exon-skipping therapies focusing on restoring dystrophin at low levels are suggested to have the potential to benefit ∼30% of all patients (Eser and Topaloğlu, 2022) and hence also struggle to treat a large cohort of DMD individuals. However, the largest challenge to the field of therapeutic genome editing might be the high cost of these drugs (Segal, 2022). Nevertheless, there is still a need for curative treatments suitable for a large group of DMD individuals, and pharmacological interventions with a "fits-all approach" are desired. This is one of the reasons the recent publication by García-Castañeda et al. (2022) and colleagues in Dr. Robert T. Dirksen's lab is attractive with its obvious transferability into drug development.

Chronically elevated levels of myoplasmic free [Ca^{2+}] is an acknowledged explanation for the observed skeletal muscle fiber deterioration and loss-of-function in DMD. Two main pathomechanisms have been proposed to cause sustained abnormalities in myoplasmic Ca^{2+} in DMD (see Fig. 1); enhanced RyR1-mediated Ca^{2+} leak from the sarcoplasmic reticulum (SR; Bellinger et al., 2009) and excessive extracellular Ca^{2+} influx via membrane tears and/or store-operated Ca^{2+} entry (SOCE; Edwards et al., 2010; Zhao et al., 2012). The Ca^{2+} sensor stromal interaction molecule-1 (STIM1) in the ER/SR membrane and the highly Ca^{2+}-selective Orai channel in the plasma membrane forms the basis for SOCE (Emrich et al., 2022).

Here, García-Castañeda and colleagues used skeletal muscle-specific tamoxifen-inducible Orai1-knockout mice crossed with mdx mice to elucidate the impact of Orai1-dependent Ca^{2+} entry in the DMD muscle pathology (Fig. 1). This approach allowed them to knockout Orai1 in the skeletal muscle of young, post-developmental mice and thus establish the effects of inducing a change into Ca^{2+} entry after the disease manifestation. Excitingly, eliminating Orai1-mediated Ca^{2+} influx in young (2–3-mo-old) mdx mice resulted in considerable functional and morphological improvements. Skeletal muscle from mdx mice lacking Orai1-mediated Ca^{2+} influx exhibited normalized intracellular Ca^{2+} homeostasis and improved muscle strength. Obliterating Orai1 expression also had a protective effect on the plasma membrane (sarcolemma) in mdx mice as it protected muscles from eccentric contraction–induced damage. Further, muscles from mdx mice

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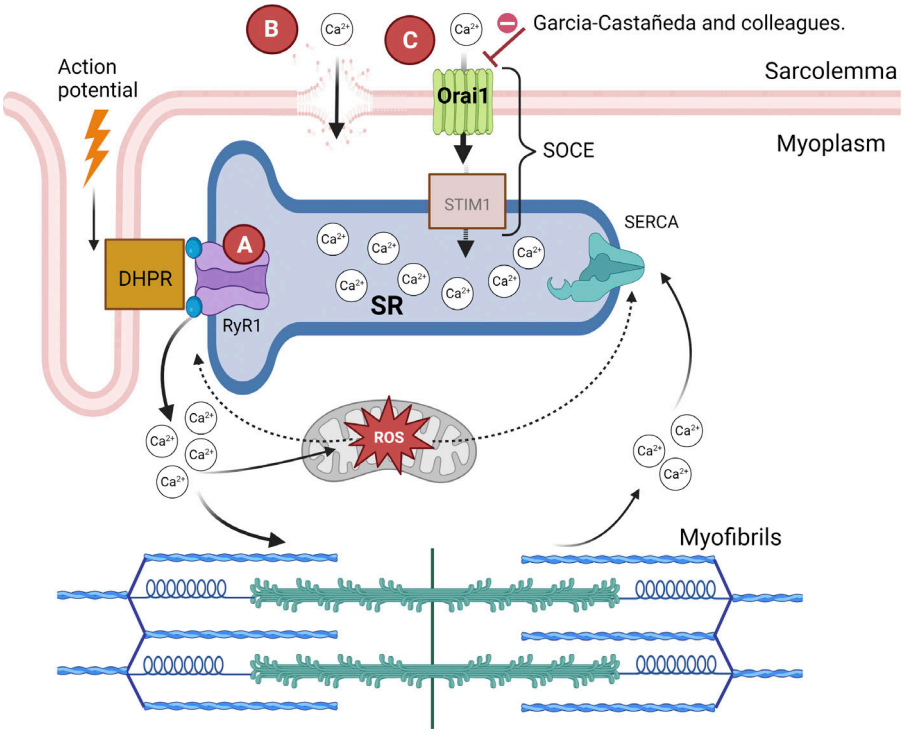

Figure 1. **A schematic illustration of the role of Ca^{2+} in the development of skeletal muscle dysfunction in DMD.** The two proposed pathways of Ca^{2+}-induced muscle dysfunction include (A) increased SR Ca^{2+} leak and extracellular Ca^{2+} entry via (B) sarcolemmal membrane damage or (C) SOCE. Dihydropyridine receptor (DHPR), ryanodine receptor 1 (RyR1), sarcoplasmic reticulum Ca^{2+} ATPase (SERCA), mitochondrial reactive oxygen species (ROS), Orai Ca^{2+} release-activated Ca^{2+} modulator 1 (Orai1), stromal interaction molecule 1 (STIM1). Figure created with Biorender.com.

with markedly reduced Orai1 expression displayed fewer histopathological hallmarks, i.e., normalized fiber cross-sectional area (CSA) and reduced muscle fibrosis. However, central nucleation was still present in muscles of mdx mice lacking Orai1 expression, which indicates ongoing regenerative processes in the mdx muscle fibers that are not reversed by restoring the intracellular Ca^{2+} homeostasis.

As addressed by the authors, many questions remain to be answered including the molecular link explaining how removal of muscle-specific Orai1-mediated Ca^{2+} influx has these beneficial effects in mdx muscle. Here the nuclear-factor κB (NfκB) and transforming growth factor β (TGF-β) signaling pathways might be of interest, given their known DMD pathomechanistic links, and that their pathway activities have been associated with Orai expression levels (Berry et al., 2018; Kang et al., 2021). Orai1 also plays an important role in maintaining Ca^{2+} homeostasis in healthy skeletal muscle, and the potential limitations of Orai1-targeted pharmacological therapy for chronic diseases, such as decreased muscle fatigue resistance with Orai1 knockout as shown by the authors need to be investigated further. Also, patients with Orai1 deficiency display muscle hypotonia and severe combined immunodeficiency coupled with autoimmunity (McCarl et al., 2009), highlighting Orai1's important role for health. Until the physiological function of Orai1 in skeletal muscle and other tissues is better understood, it is still too early to suggest it as a promising drug target in humans. Drug target or not, Orai1 is also expressed in a wide variety of tissues, which stresses that a potential future intervention needs to be tissue specific and carefully dosed to avoid detrimental and dangerous side-effects. Another DMD-related aspect that would be interesting to assess is a potential Orai1 involvement in the DMD-induced diaphragm and cardiac dysfunction. Although DMD clinically manifests as progressive muscle weakness, it is the respiratory failure and heart problems (often develop in the form of dilated cardiomyopathy) that ultimately causes the premature death. The diaphragm is a skeletal muscle with a similar cytoskeleton, extracellular matrix, and excitation–contraction (EC) coupling as the limb muscles examined by García-Castañeda and colleagues, and hence one could speculate that it is likely that the removal of Orai1-mediated Ca^{2+} influx has beneficial effects in DMD muscle. On the other hand, there is more uncertainty whether eliminating Orai1-mediated Ca^{2+} influx in cardiac muscle would have any effect given that cardiac muscle is designed to handle Ca^{2+} influx as its EC coupling, and thus every heartbeat, is initiated by Ca^{2+} influx via the voltage-gated Ca^{2+} channel (Cav2.1, L-type Ca^{2+} channel). Hopefully this and additional aspects of Orai physiology and pathophysiology in striated muscle will be addressed by the Dirksen laboratory or others in the near future. Nevertheless, the results of García-Castañeda et al. (2022) demonstrate an important role of enhanced Orai1-mediated Ca^{2+} entry in exacerbating the dystrophic phenotype of mdx mice, rendering Orai1 a potential therapeutic target with a "fits-all approach" for the treatment of DMD. On that note, it is promising that there is a continuous development of Orai1 inhibitors (Azimi et al., 2020) for various indications, and some have entered clinical trials with DMD potentially included in the near future.

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